displacements of the same sign and order of magnitude as the stable lines of groups *a* and *b*.

Tests of recently published wave-lengths of calcium and manganese show that they are contaminated by the disturbing influence of pole effect and it appears probable that little of the new work in wave-length determination is sufficiently free from this influence to meet the more and more exacting demands of the present and immediate future, for high accuracy in wave-length measurement.

We recommend that light be taken from a narrow equatorial zone of a 4 to 5 fold enlarged image of an iron arc of the Pfund type 12 mm. long carrying a current of 5 amperes. With such an arc the exposure time for the region λ 5600 to the violet is not excessive; for the region, λ 5600 to λ 6000, it is somewhat long when very high dispersion is required; but in this region the International arc is entirely lacking in stable lines and no element yields a sufficient number of lines of good quality here; from λ 6000 to the red the International Secondaries belong to group *b*. They are free from perturbing influences and for them any form of iron arc may be used as the source.

To obtain dependable wave-lengths of other elements the necessary preliminary is an examination for pole effect. If it is found to be present, a method for its elimination should be worked out and applied before attempting the wave-length measurements. As the method of the elimination depends upon the element, the problem of wave-length determination is no longer one of mere routine but offers opportunities for a real investigation.

[1] *Smithsonian Physical Tables*, p. 172.

[2] *Trans. Int. Union Coöp. Solar Research*, **4**, (59).

[3] St. John, C. E., and Babcock, H. D., *Mt. Wilson Contrib.*, No. 106; *Astroph. J., Chicago*, **42**, 1915, (231–262).

[4] St. John, C. E., *Mt. Wilson Contrib.*, No. 123, pages 11 and 27; *Astroph. J., Chicago*, **44**, 1916, (311–341), pages 321 and 337.

ON THE PRESENCE OF ALBUMOSES IN EXTRACTS OF THE POSTERIOR LOBE OF THE HYPOPHYSIS CEREBRI

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In recent years some very definite statements have been made in respect to the chemical nature of the active principle or principles of the hypophysis cerebri (pituitary gland). Among the claims advanced none are more sharply defined than those published by H. Fühner[1] on

behalf of the chemists of the research laboratory of the Farbwerke-Hoechst Company.

From extracts of the posterior lobe of the hypophysis, which had been freed of coagulable proteids,[2] these investigators obtained a mixture of crystalline sulphates of high physiological activity which was quite unjustifiably named 'Hypophysin.' What misconceptions may arise in connection with the use of this term 'hypophysin'—a designation for an unknown number of substances—may be seen when we read in a treatise on organotherapy that "Hypophysin is the chemically active pure posterior pituitary hormone, marketed as a sulphate."[3]

Further research enabled the chemists referred to to separate their 'hypophysin' into four unnamed crystalline fractions which, in respect to physical and chemical properties, are easily distinguished, the one from the other. These fractions are described in the following words:

1. A colorless, well crystallized sulphate which easily dissolves in water with neutral reaction and is difficultly soluble in alcohol, acetone and ethyl acetate. It is optically active (lævo-rotatory, $[\alpha]_D = -54.02°$) and carbonizes without melting when heated to a high temperature. The Pauly and biuret reactions are positive. With picric acid this substance forms a salt difficultly soluble in water.

2. There was also obtained a substance yielding a well crystallized, colorless sulphate which dissolves in water with faint acid reaction and is likewise difficultly soluble in alcohol, acetone, ethyl acetate, etc. The optical rotatory power of this preparation is $[\alpha]_D = -27.17°$; it decomposes when heated to 198–200°C. and gives the Pauly and biuret reactions. Contrary to the substance described under (1) this compound forms a picrate easily soluble in water. If this substance is brought into contact with alkalies a volatile amine base is at once liberated.

3. A third substance was isolated in the form of a crystalline, faintly yellow sulphate which, to be sure, is present in only very small amount. It is easily soluble in water and methyl alcohol with faintly acid reaction, and difficultly soluble in absolute alcohol, acetone and ethyl acetate. It turns the plane of polarized light to the left; its rotatory power is $[\alpha]_D = -39.25°$. On heating the substance decomposes at 185–186°C. The Pauly and biuret reactions are positive. With picric acid is obtained a salt easily soluble in water.

4. The mother liquor remaining after the fractional precipitation of the three substances just described yields, on cautious evaporation in a vacuum, a brittle, glassy, hygroscopic mass which dissolves easily in water and methyl alcohol, with difficulty in ethyl acetate and acetone. The solution of this substance shows an optical rotatory power of $[\alpha]_D = -21.26°$ and gives the Pauly but not the biuret reaction. Recently it has been found possible to

isolate also from the mother liquors a yellow, crystalline, neutral substance which dissolves easily in water and alcohol, with difficulty in ether, acetone and ethyl acetate. The preparation in aqueous solution rotates the plane of polarized light to the right ($[\alpha]_D = +5.99°$) and gives neither the Pauly nor the biuret reaction. The decomposition temperature is 95 to 96°C. The preparation gives with picric acid a compound difficultly soluble in water.

'Hypophysin' (the mixture of substances) is stated by Führer to represent the physiological activity of the posterior lobe in respect to blood-pressure, respiration and uterine contractility and the sum of the actions of the four crystalline fractions equals that of the undifferentiated hypophysis.

Führer's conclusions as to the pharmacological action of the four fractions are as follows:[4]

Fraction 1 has only a slight action on the respiratory apparatus and the uterus, but shows the typical action of 'hypophysin' on blood-pressure.

Fraction 2 has a pronounced action (*ausgeprägte Wirkung*) on blood-pressure, on respiration and on the uterus.

Fraction 3 behaves qualitatively like fraction 2 but has a more marked stimulating action on the uterus.

Fraction 4 (mother liquor and crystalline part) has an action on the uterus equivalent to that of fraction 3 but only a very slight action on blood-pressure and on respiration.

From his experiments Führer draws the conclusion that the uterus-stimulating power of the hypophysis, which is, practically speaking, the most valuable property of extracts of the gland, resides, not in one, but in various (*verschiedene*) constituents of the organ, the condition being analogous to that found to hold for ergot.

From a chemical point of view, it is especially noteworthy that fractions 1, 2 and 3 give the biuret reaction as well as Pauly's reaction and that all three are laevo-rotatory; fraction 1, physiologically the least active, has the highest rotation, $[\alpha]_D = -54.02°$. It is easily demonstrated, we believe, that one or more albumoses (or polypeptids if the term is preferred) are present in the first three of these fractions and no doubt also in the fourth. We have found, as will be shown in a subsequent paper, that substances of the nature of albumoses can be isolated from many organs, It is these substances, which are themselves inactive, that give the biuret, the Pauly and other reactions of the so-called isolated principles and that account for their laevo-rotation.

The following analysis of one of the commercial preparations of the posterior lobe of the hypophysis is offered in support of our contention

that all those fractions of 'hypophysin' which give the biuret reaction contain albumose. The extract called 'Pituitrin' (Parke, Davis & Co.) was used first because it could be purchased in large quantities ($\frac{1}{2}$ oz. bottles).

The contents of 10 bottles, approximately 150 cc., were extracted three times with ether to remove a preservative (chloretone) and then concentrated under an electric fan on the water bath to a volume of 10 to 15 cc. A small amount of flocculent material which separated was removed by filtration, the filtrate was diluted with an equal volume of absolute alcohol and a solution of lead acetate was added, which induced a very slight precipitation. Addition of ammonia to faint alkalinity did not materially increase the precipitate, from which it was concluded that no appreciable amount of native proteid could be present, and that no advantage would accrue from the use of basic lead acetate, as in the procedure which will be described in a subsequent paper. An albumose, especially if of secondary nature, would escape precipitation by ammoniacal lead acetate. The alcoholic filtrate was freed from lead with sulphuric acid and the filtrate from the lead sulphate was concentrated to a small volume (a few cc.) and treated with an equal volume of saturated ammonium sulphate solution. The resulting flocculent precipitate, which was not very abundant, was washed with half-saturated ammonium sulphate solution, dried *in vacuo*, dissolved in water and again precipitated with an equal volume of saturated ammonium sulphate solution. The precipitate was again washed with half-saturated ammonium sulphate solution and treated with an excess of barium hydroxide. After filtering off the barium sulphate, the filtrate was heated on the water bath until all the ammonia had been expelled and the excess of barium was removed with sulphuric acid. The filtered solution was then concentrated to a very small volume and dropped into absolute alcohol, ether being added until no further precipitation was produced. The substance here thrown out, which was small in bulk (0.010 g.), was of less interest to us than the fraction presently to be described. It gave the Millon and biuret reactions, as well as that of Pauly. Knoop's reaction for histidine was negative.

The filtrate from the half-saturation with ammonium sulphate as described above was saturated with finely powdered ammonium sulphate, which caused the appearance of a gummy precipitate so characteristic of albumoses when treated in this manner. The precipitate was filtered off, washed with saturated ammonium sulphate solution, dissolved in water and treated with barium hydroxide in the usual manner. The solution, freed from ammonia and the excess of barium, was concentrated to a very small volume and dropped into absolute alcohol, the precipitation being completed with ether. The substance thus thrown out was collected as completely as possible and dried *in vacuo;* 0.031 g. in 1 cc. of water in a 0.5 dcm. tube showed a rotation of $-1.38°$, whence $[\alpha]_D = -89°$. When the substance was reprecipitated with hot absolute alcohol its specific rotation was found to be $[\alpha]_D = -77.6°$.

As to its properties, the substance must be classed with the secondary albumoses. It has been shown to be precipitated by saturation of its solution with ammonium sulphate. It is non-coagulable on boiling, gives the biuret reaction (hemi-biuret) very beautifully, as also Pauly's reaction, while Knoop's bromine reaction for histidine is entirely negative. This negative result with Knoop's reagent excludes the presence of histidine as an admixture in our substance.[5] Picric acid added to an aqueous solution gives a precipitate. The addition of Millon's reagent gave rise to a slight turbidity, but on boiling the characteristic red color was not obtainable. In this respect there was entire agreement with a secondary albumose which we have isolated from the mucosa of the small intestine. The ninhydrin reaction was positive when made in the usual manner but we are confident that this is due to the fact that adherent amino acids were not entirely removed. To do this would have required several reprecipitations with ammonium sulphate.

The albumose here described was found to have a quite negligible action when tested with the virgin cat's uterus.

A secondary albumose which was prepared by digesting fresh thyroid glands of the pig behaved in every respect like the above substance. Its specific rotation was found to be $[\alpha]_D = -88.1°$, while that of the pituitary albumose varies from $-78°$ to $-89°$.

We come now to the filtrate from the complete salting out with ammonium sulphate. This was freed from ammonium sulphate in the usual manner. Reduced to a small volume, the solution was dropped into absolute alcohol, the precipitation being completed with ether as with the preceding substances. The material thus obtained was readily soluble in water and gave the usual response of pituitary extracts when tested on the isolated uterus of the virgin guinea pig. It also still gave the Pauly and the biuret reactions, though with greatly lessened intensity as compared with the original solution. It fails to give Knoop's reaction for histidine so that we must conclude that this amino acid does not exist as such in any considerable amounts, if at all, in pituitary extracts. The rotation of 0.0381 g. in 1 cc. of water in a 0.5 dcm. tube was found to be $-0.52°$, from which $[\alpha]_D = -27.4°$. We have here a substance which may be compared with respect to physiological activity, rotation and chemical tests with Führer's 'hypophysin' fractions 2 and 3. The solution used in the polarizing tests was saturated with powdered ammonium sulphate and again the characteristic precipitate of albumose was produced, though, naturally, it was not abundant this time. Evidently we have here the remnant of albumose which remained in solution after the first saturation with ammonium sulphate. If we were to apply this salting out to larger quantities of pituitary extract it is possible that more or less of a true peptone would be found in the filtrate. Certainly, there always remains in the filtrate, even after two

saturations, a substance which gives a pink biuret reaction. Only more extended research can show to what extent this is albumose and to what extent peptone.

We believe that there is no mystery attaching to the constituents of pituitary extracts that have been shown to give positive Pauly and biuret reactions *and a negative Knoop's bromine reaction.* These constituents must be classed as albumoses (and even peptones) and the German chemists are to be congratulated if they have obtained them in the form of physiologically active crystalline sulphates, as has been stated on their behalf by Führer, even though they have failed to recognize their proteid nature.

We have made qualitative tests with other commercial pituitary extracts (Armour's Pituitary Liquid, and Solution Pituitary Extract, Mulford) and have found, as was to be expected, that albumoses can be salted out from all of them. The amount of proteid material present varies considerably in these preparations—one of them (Armour's Pituitary Liquid) appears to have only a trace of that form of proteid (coagulable proteid plus primary albumoses) which gives a precipitate with potassium ferrocyanide and acetic acid and to have practically all its biuret-yielding substance in the form of secondary albumose.

As to the total amount of biuret-yielding material present in the extract analyzed—and by this we mean the substance or substances that give the biuret reaction *immediately at room temperature* (and not, as histidine gives it, after heating)—we believe that we are close to the truth when we say that it cannot be far below 10% in weight of the total solid matter. The dry residue from five bottles of extract (73 cc.), exclusive of chloretone, was found to be 0.415 g. The amount of albumose, primary (?) and secondary, recovered from ten bottles (in the analysis described above), with a dry residue of 0.830 g., was approximately 0.050 g. The losses, at a conservative estimate, could hardly have been less than 0.025 to 0.030 g. On this basis we should have had, in the specimen analyzed, close to 10% of biuret-yielding material.

It may be of interest to state here that when the dry residue of five bottles (0.415 g.) *was heated in a boiling water bath for one hour in 10 cc. of 25% hydrochloric acid the biuret reaction*[6] *disappeared entirely* but the Pauly reaction for histidine was still obtained. The disappearance of the biuret reaction after boiling with hydrochloric acid can only be interpreted as due to hydrolysis of our albumose.

After having completed our examination of the American pituitary preparations we learned that the 'hypophysin' of the Hoechst chemists could be ob-

tained in this country. We accordingly purchased two hundred 1 cc. ampullæ of this product, which is described on the labels as a "sterile solution, 1:1000, of the isolated active substances from the glandula pituitaria." We did not inquire if a preservative is used in its preparation, as the presence of a substance of this character would hardly interfere with the isolation of a proteose.

The residue from ten ampulles, as obtained by evaporation at a low water-bath temperature under an electric fan and subsequent drying over sulphuric acid, amounted to 0.0154 g. The dry residue from the two hundred 1 cc. ampulles would therefore have weighed 0.3080 g. One hundred and ninety cubic centimeters, that is to say, the total quantity of solution with the exception of the 10 cc. used for the estimation of the dry residue, were concentrated on the water bath under the fan to a volume of 2.5 cc.[7] and saturated with finely powdered ammonium sulphate. The characteristic gummy precipitate of salted-out albumose immediately collected on the stirring rod and on the sides of the tube containing the solution. The precipitate was washed with saturated ammonium sulphate solution, decomposed with barium hydroxide and the freed albumose was precipitated as a "sulphate" with absolute alcohol and ether in the manner already described. Dried over sulphuric acid, the albumose thus obtained weighed 0.017 g. The reactions were those already described—a beautiful pink biuret, a positive Pauly and a negative Knoop reaction. Potassium ferrocyanide and acetic acid also failed to give a precipitate, showing that coagulable proteids and primary albumoses were not present.

The ammonium sulphate filtrate from the gummy albumose precipitate still gave a fine pink biuret reaction, as was the case also with all the American preparations under the same conditions. The addition of a drop or two of a very concentrated solution of trichloracetic acid to this filtrate caused an immediate precipitation of gummy droplets. These give the biuret reaction with great intensity and represent a further yield of albumose with a probable admixture of peptone and traces of other substances. It may be stated in this connection that the ammonium sulphate filtrates of our American preparations also give with trichloracetic acid a precipitate which is indistinguishable in its reaction from that obtained with hypophysin. A certain amount of albumose or peptone still remains in these ammonium sulphate filtrates even after the use of trichloracetic acid.

The Hoechst preparation is no doubt a clean product and certainly contains less dry residue than the products prepared in this country. We have seen that the dry residue of ten 1 cc. ampulles of hypophysin was 0.0154 g. The dry residue of ten 1 cc. ampulles of Armour's Pituitary Liquid was 0.0242 g. and in its relatively smaller content in proteoses this prepration more nearly approaches hypophysin than any other examined by us. It is to be understood that we are not criticizing these products because they happen to contain more or less albumose.

This in itself is of no consequence, as this albumose does not appear to be toxic.

The points that we wish to emphasize are these:

1. Carefully prepared commercial extracts of the posterior lobe of the hypophysis contain albumoses.

2. Hypophysin, stated to be a mixture of the "isolated active substances of the pituitary gland," is likewise contaminated with albumoses.

3. All claims in respect to the isolation of pure principles, as made by the Hoechst chemists, must be looked upon, in view of our findings, as being without foundation.

Other considerations also lend support to the last statement.

One who is familiar with the high activity for the virgin uterus of fresh extracts of the hypophysis can only agree with Fenger when he asserts that the as yet unknown constituent of this gland which affects the uterus so powerfully cannot be less potent than β–imidoazolylethylamine, and may be even more powerful. Fenger says that an acidulated methyl alcohol extract of the posterior lobe of the hypophysis, for which no claim to chemical purity can be advanced, "showed a uterine-contracting power somewhat stronger than pure β–I."[3] If we examine the tracings given by Fühner in his experiments with the Hoechst products, experiments in which quantities varying from 0.05 to 0.5 mgm. were tested on the guinea pig's uterus in a 100 cc. bath of Locke's solution, it will be seen that these products are much weaker than β–imidoazolylethylamine. Here again is evidence that the crystalline salts of the Hoechst chemists represent mixtures of active and inactive principles and not pure chemical individuals.

Further evidence that Hypophysin does not consist of chemically pure principles is given by the pharmacological tests made with it in this laboratory. Dr. D. I. Macht has kindly compared the oxytocic strength of the preparation with that of Armour's Pituitary Liquid, this having been selected from among the American products because it most nearly approaches Hypophysin in respect to dry matter and a low albumose content. He reports that the Armour product, which makes no pretense of being a pure chemical principle, is "several times more powerful in its action on the virgin uterus of the guinea pig than Hypophysin." There is no reason to assume that the Hypophysin used in Dr. Macht's tests had lost any of its original strength as the labels on the packages give no hint of instability or loss of strength with time.

The question naturally arises whether the albumose or other proteose here shown to be present in all active pituitary extracts is not itself the uterine stimulant. The secondary albumose which was isolated by us

from "Pituitrin" was practically devoid of an oxytocic action, as has already been stated. Investigations on the bio-chemistry of the intestinal and gastric mucosa which we hope soon to publish also lend no support to the theory that pituitary extracts contain an active albumose. We have prepared a water-soluble powder from this mucosa which is highly active for the guinea pig's uterus (1:1,000,000) and for the intestinal strip (1:250,000), which induces a distinct rise of blood-pressure in the cat, and which in respect to its chemical reactions, its behavior towards ammonium sulphate and polarized light, *is indistinguishable from a diluted pituitary extract.* The similar behavior in these several respects of gastric and intestinal 'motiline' solutions and pituitary extracts first led us to suspect that these latter also contain albumoses. Now, in the case of these intestinal preparations, we have had sufficient material on hand for the application of purification processes. *We finally emerged with a secondary albumose which was entirely devoid of oxytocic, pressor, depressor or secretory action.* It is this experience, together with our discovery than an inactive albumose can be prepared from the ordinary pituitary extracts, as already stated, which fortifies us in our belief that the proteoses of the gland have nothing whatever to do with the physiological activity of the organ.

It is not our purpose to consider here the literature[9] pertaining to the 'peristaltic hormones' that are known to occur in almost all, if not all, organs of the body, It is worthy of note, however, that an extract of the gastric or intestinal mucosa can be prepared, as we have already stated, which has a pressor action for the circulation and a marked oxytocic power in a concentration of 1:1,000,000. This powerful action points strongly to the conclusion that here also, as in the case of pituitary extracts, we are dealing with a motiline which, in a state of chemical purity, would be fully as active as β–imidoazolylethylamine. And this again leads us to the supposition that the oxytocic principle (or motiline) of the hypophysis is not a hormone or substance specific to this organ, but is rather a widely distributed substance, everywhere the same, which may have its origin in the various tissues, in the gastric or intestinal mucosa, or which may be absorbed as such from among the products of digestion. We hope that our discovery of the contaminating and difficultly separable proteoses in physiologically active extracts will pave the way to the solution of these problems.

We cannot conclude this communication without adding a few words in regard to the presence of proteoses in the various tissues of the body. Proof of their existence in pituitary extracts has been given and reference has been made to their presence in gastric and intestinal extracts. By

the use of certain methods which will shortly be described in detail we have found that a secondary albumose (to name only a single proteose which is sharply differentiated from all native proteids and primary proteoses) can be isolated in small amounts from all of the cellular tissues of the body thus far examined. Skeletal muscle appears to contain albumose in the smallest amount, gastric and intestinal mucosa contain it even after four days' starvation, *much more during digestion of a meat meal*, while organs like the thyroid gland contain much more, weight for weight, than skeletal muscle. We have not as yet been able to isolate definitely a true proteose of any sort from the plasma of the blood, though able to show that the cellular elements of the blood yield a readily demonstrable amount of albumose.

It was not originally our purpose to study these proteoses or to isolate them from the various tissues, but finding them always present in our final products whenever we attempted to isolate certain 'hormones,' such as the intestinal motiline and secretine, even when our methods of treating the tissues could not have produced them, we were forced to undertake a study of methods for their separation from the hormones. A future communication in these PROCEEDINGS will deal with this question.

Conclusions—1. Secondary albumoses and possibly peptones (or polypeptids if the term is preferred) were found to be present in all of the therapeutically used extracts of the posterior lobe of the hypophysis cerebri that were examined. To what extent the proteose content of the gland may have been increased by autolysis or by processes incidental to the manufacture of the extracts it is impossible for us to state. We believe, nevertheless, that the perfectly fresh, bloodless glands yield proteoses, inasmuch as we have actually isolated such substances from the thyroid gland and other organs when taken from the animal immediately after bleeding it to death.

2. The 'Hypophysin' of the Farbwerke-Hoechst Company is not, as claimed for it, "a solution of the isolated active substances of the pituitary gland" but a mixture of albumoses (and possibly peptones) with varying and unknown amounts of active and inactive constituents of the gland. The albumoses present in 'Hypophysin' account fully for the chemical reactions (such as the biuret and the Pauly reactions and the left-handed rotation) which are stated to characterize the pretended active principles. The albumoses as separated from pituitary extracts are devoid of action upon the uterus. In view of the facts here presented it must be evident that the active principles of the hypophysis cerebri have not yet been isolated as chemical individuals.

[1] Fühner, H., *Zs. ges. exp. Medizin, Berlin*, **1**, 1913, (397).

[2] "Vollständig von Eiweiss befreite Auszüge aus den Hinterlappen von Rinderhypophysen." Fühner, *loc. cit.*, p. 399.

[3] Harrower, H. R., *Practical Hormone Therapy*, New York, 1914, p. 460. A Glossary of Terms.

[4] Fühner, *loc cit.*, p. 443.

[5] Cf. Aldrich, T. B., *J. Amer. Chem. Soc., Easton, Pa.*, **38**, 1915, (203).

[6] The biuret reaction was made at room temperature. Unfortunately we did not apply heat as is done when making this test for histidine. This substance was no doubt present, having been set free from the albumoses by hydrolysis, as is shown by the positive Pauly test.

[7] This also contained the redissolved residue from the 10 cc. used for the estimation of dry matter.

[8] Fenger, F., *J. Biol. Chem., Baltimore*, **25**, 1916, (417).

[9] Cf. Ott, Enriquez and Hallion, Zuelzer, Weiland, Köhler and others.

ON THE RÔLE OF THE THYMUS IN THE PRODUCTION OF TETANY

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In a number of experiments on larvae of *Amblystoma punctatum* and *A. opacum* the influence of thymus was studied. It was found that the effect of thymus upon these animals is not exactly similar to its effect on tadpoles of frogs or toads. With regard to this difference the most conspicuous and important effect of the thymus feeding in Salamander larvae is the occurrence of severe tetany in the thymus-fed larvae.

Up to the present, 67 specimens of *A. punctatum* and *A. opacum* were fed exclusively on Thymus after they had reached an age of about six to fourteen days. In each single individual tetany was produced.

For several days previous to the occurrence of the acute attacks, the animals are less active and their appetite is diminished. The acute stage appears in two forms: a mild form and a severe form. The mild form manifests itself by clonic convulsions of the hind limbs and the tail, while the severe form consists in clonic convulsions of the entire system of muscles. In the beginning of the tetany period, the attacks exhibit the characteristics of the mild form, then become severe and towards the end of the entire period again are mild. Each acute attack is followed by a tonic spasm of the entire body, during which the body is stiff for a short time. After several acute attacks the hind limbs become permanently twisted and stiffened and are to some extent paralyzed. Later on the forelegs and in severe cases even the neck and spinal cord become paralyzed.